# Mapping interactions between the HIV-1 genome and human lysyl-tRNA synthetase

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## Background

- As of 2013, there were approximately 35 million people living with an HIV-1 infection.
- Although combination drug therapy has been successful in prolonging the life of AIDS patients, HIV-1 is a highly mutagenic virus and readily develops drug resistance.
- The purpose of this study is to use RNA structure-probing techniques to map the interaction between human lysyl-tRNA synthetase (LysRS) and HIV-1 genomic RNA.
- This will help better understand this key viral regulatory mechanism, which may contribute to the future design of new therapeutics.

Global Report: UNAIDS report on the global AIDS epidemic 2013

M. Nijhuis, S. Deeks and C. Boucher, *Current Opinion in Infectious Diseases,* vol. 14, no. 1, pp. 23-28, 2001.

## HIV-1 life cycle

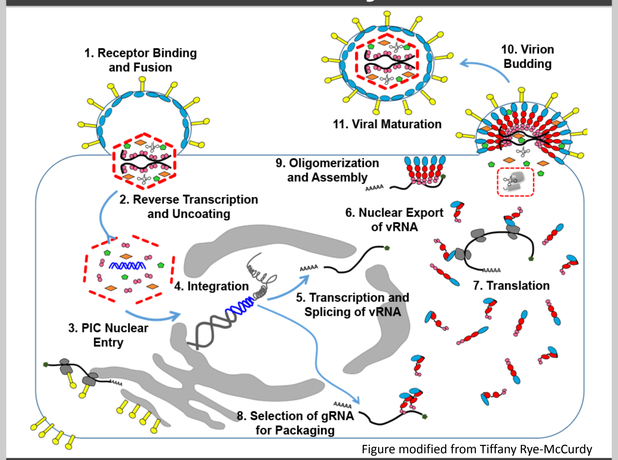
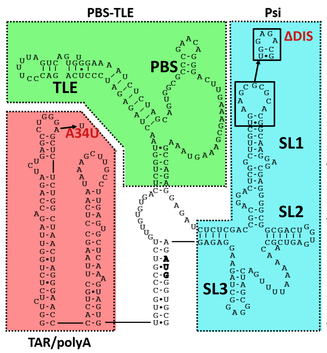



Figure modified from Tiffany Rye-McCurdy

## 5´ UTR regulates RT initiation

The 5’ untranslated region (5’ UTR) of the HIV-1 genomic RNA is highly-structured and conserved. It also regulates many key viral functions. This project focuses the mechanism by which the 5’ UTR regulates reverse transcription (RT) initiation.

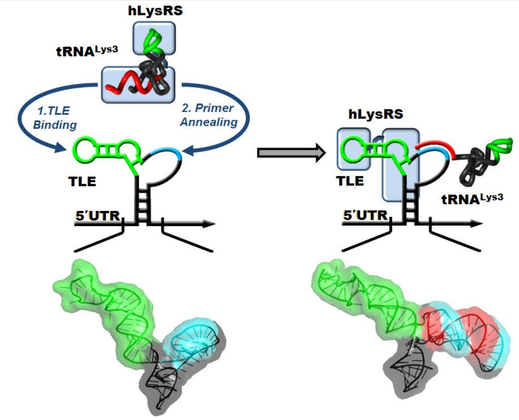



| RNA | LysRS $K_d$ (nM) |
|---|---|
| $tRNA^{Lys3}$ | 407 ± 33 |
| PBS-TLE | 383 ± 18 |
| PBS + Psi-SL1 | 281 ± 25 |
| PBS + Psi-SL1-SL2 | 85.4 ± 5.4 |

- LysRS is a key factor in RT regulation.
- Fluorescent anisotropy (FA) binding experiments show that high affinity LysRS binds requires Psi elements.
- 5’ UTR mutants used in this study are denoted in red.

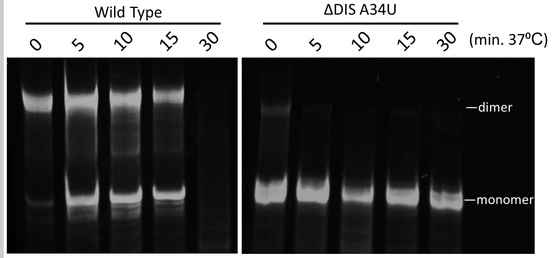
*Jones, Cantara, Olson, Musier-Forsyth. PNAS **111**:3395-3400 (2014).*

## LysRS-viral genome interaction




The 5’ UTR includes the primer binding site (PBS), which is the site of RT initiation. HIV-1 uses a host cell $tRNA^{Lys}$ to prime reverse transcription, and our lab has previously shown that LysRS binds to a tRNA-like element (TLE) of the 5’ UTR to facilitate this process.

## 5’ UTR dimerization mutant is monomeric

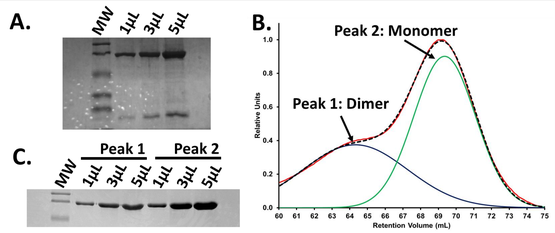



Mutant and wild type RNAs were *in vitro* transcribed, gel purified and butanol extracted. They were then folded and analyzed for homogeneity showing that the dimerization mutant (ΔDIS A34U) is monomeric and homogeneous.

**Folding Conditions**

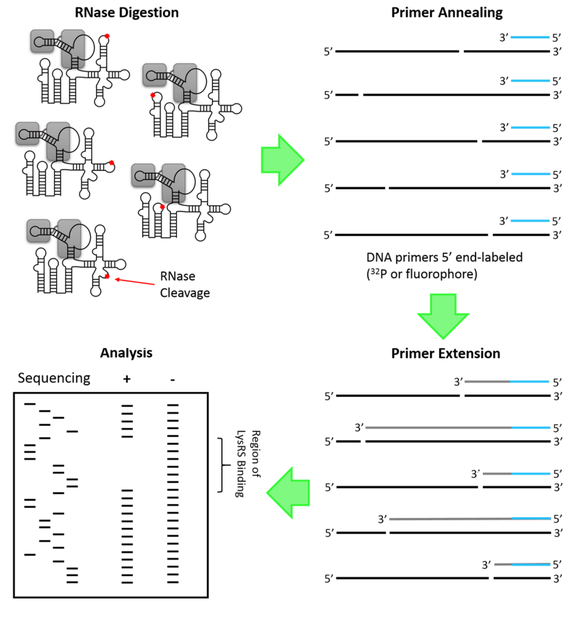
1. 2 min. 80ºC
2. 2 min. 60ºC
3. Add $Mg^{2+}$
4. Incubate at 37ºC

## LysRS 3M is largely monomeric

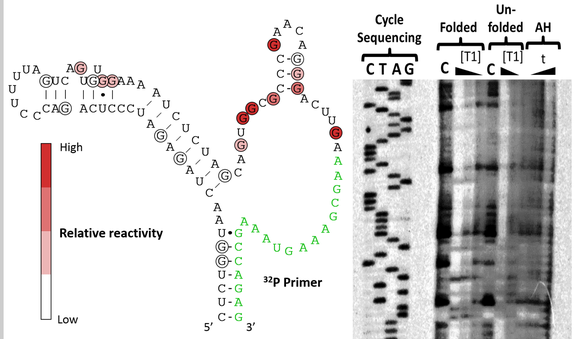


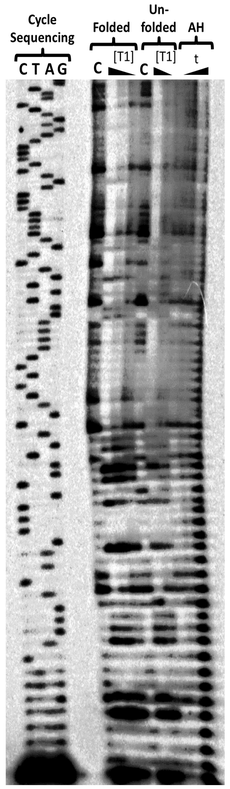

LysRS triple mutant (3M) was purified using His-Tag $Ni^{2+}$ affinity, and analyzed for purity using SDS PAGE **(A)**. Size exclusion using a Superdex 200 Increase 10/300 GL column (GE LifeSciences) showed two peaks **(B)**. These were deconvoluted, and their fractions were isolated and analyzed using SDS PAGE to show that both contained solely LysRS **(C)**. Thus, the peaks must be the monomer and dimer, indicating that the sample is primarily monomeric.

## RNase protection experimental scheme



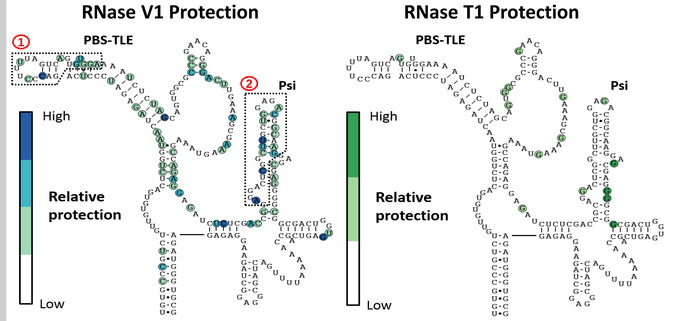

## RNaseT1 experiments agree with secondary structure

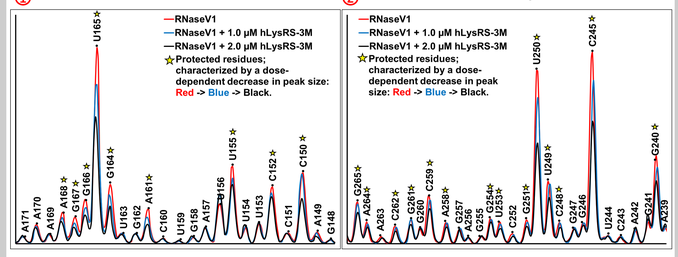



- RNase digestion experiments on the PBS region of the 5’ UTR were performed to optimize cycle sequencing, RNase concentrations, and primer extension.
- The experiments were done on folded and unfolded RNAs at varying RNase concentrations, and alkaline hydrolysis (AH) was used to help map nucleotide position.
- RNase T1 cleaves at single stranded guanosine residues, and the cleavage map above shows that the data agree with the accepted secondary structure of the PBS region.

## RNase protection results suggest LysRS binds to PBS and Psi regions




- RNase V1 and T1 protection experiments were analyzed using capillary electrophoresis (CE) to make the data more quantifiable.
- These RNases were chosen to account for regions of high secondary structure (RNase V1 cleaves DS residues), enrichment of single-stranded G residues in Psi (RNase T1) and single-stranded pyrimidines in the TLE (RNase A).
- These experiments were done with increasing concentrations of LysRS to observe the dose-dependent protection seen in the traces above.
- The protection data suggest that LysRS binds to both the TLE and stem loop 1 (SL1) residues.
- Further replicates of these experiments will be required to validate the results depicted here. Experiments with RNase A are also underway.

## Conclusions and Future Directions

**Optimization nearly complete**

- Sample homogeneity has been verified, and cycle sequencing and primer-extension/CE are functioning properly.
- RNase concentrations still need to be optimized to improve reproducibility of the data.
- An additional, more central primer will be used to obtain better data from the TAR/polyA region.

**Conclusions**

- The current data imply that LysRS interacts significantly with SL1 of Psi as well as PBS-TLE.

**Future directions**

- Once the conditions are optimized, this technique will be readily applied to footprint any protein that binds to this RNA (e.g. plans are underway to footprint HIV-1 reverse transcriptase and human RNA helicase A).